

テンサイ花器(球果)に対する炭そ病菌の感染過程

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Histopathology of Sugar Beet Flowers and Seed Balls Infected with *Colletotrichum dematium* f. *spinaciae*

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Abstract

The infection process of sugar beet flowers and seed balls by *Colletotrichum dematium* f. *spinaciae* was studied using light microscope and scanning electron microscope. When infection occurred at early stage of flowering, germ tubes produced appressoria and subsequently infection pegs penetrated the cuticle 3~4 days after inoculation. Mycelia spread both inter- and intracellularly. Infected cells eventually collapsed, and lesions became visible about 5 days after inoculation. Mycelia were found not only on the surface of blackened seed caps or pericarps, but also in the inner space of the pericarp. In severe infection, mycelia also found between the seed coats and cotyledons. When infection occurred at the late flowering stage, mycelia were frequently observed on the seed surface, but less frequently inside the seed balls. These hyphae were found in the periphery of the apical pore or inside the seed coat. The results indicate that this pathogen is seed-borne.

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Key words: sugar beet, anthracnose, infection process, scanning electron microscopy.

INTRODUCTION

Since 1981 when an outbreak of anthracnose disease occurred in sugar beet fields in Japan, *Colletotrichum dematium* f. *spinaciae* (Ellis et Halst.) von Arx became a significant problem in seed production of sugar beet³⁾. The pathogen also causes damping-off, petiole and leaf spot of sugar beet for root crop. Infestation of the seed production fields resulted not only in yield loss of seeds but also in deterioration of seed quality due to contamination.

A seed ball of sugar beet is a fruit consisting of three parts, *i.e.* seed cap, true seed and pericarp. True seed, which is the seed from the botanical point of view, is lying within dead corkey tissue (pericarp). Seed cap covers the upper part of the true seed (Fig. 1)¹⁾.

Although varietal differences in susceptibility to the pathogen⁵⁾, survival of the pathogen in soil⁴⁾ and chemical control measures^{2,10)} have been described, establishment process of the pathogen in flower and seed balls has not been studied in detail.

The purpose of the present work was to observe conidial germination, penetration by infection peg, and establishment of *C. dematium* f. *spinaciae* in the sugar beet flower and seed balls using light microscope (LM) and scanning electron microscope (SEM).

MATERIALS AND METHODS

Inoculation. Two lines of sugar beet, TK-76-49/2 mm-CMS and TK-76-49/2 mm-O, were used throughout the experiments. These lines are susceptible to anthracnose⁵⁾. The

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sugar beet, as a biennial crop, requires usually two years for seed production. Mother beet plants or stecklings were grown from seeds during the first year. Stecklings were planted in pots, placed in a greenhouse and allowed to bolt for seed production after an overwinter storage. Just before flowering, inflorescence was bagged for each line separately to prevent spontaneous cross pollination. Pollination was done by exchanging bags covering inflorescences.

Inoculation was made by spraying conidial suspension of *C. dematium* f. *spinaciae* (Isolate, CS-22M) on inflorescence of the CMS line 7 (stage 1) or 21 days (stage 2) after pollination. Stages 1 and 2 correspond to periods of cotyledon differentiation and maturation of seed organs, respectively⁹⁾. Flowers or seed balls were harvested 2, 3, 4, 5, 7, 11 and 14 days after inoculation.

Tissue preparation for LM. Samples were fixed in FAA for 24 hr, dehydrated through an ethanol series and embedded in paraffin. They were cut and stained with 0.1% cotton blue in lactophenol.

Tissue preparation for SEM. Samples were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1.5 hr at room temperature, post-fixed in 2% osmium tetroxide in the same buffer for 12 hr at room temperature, dehydrated in an ethanol series for 30 min per step and dried in a critical-point dryer. They were mounted on aluminum stubs and coated with gold using an ion coater. Observations were made at 10 kV under JSM-T200 SEM.

RESULTS

Infection process of sugar beet anthracnose

Observations were focused on infection process on the seed cap since, in most cases, symptoms developed first on the seed cap and then spread over the whole seed.

Stage 1 (Infection at the early stage of flowering)

Conidia germinated and produced appressoria on the cuticle of the seed cap two days after inoculation (Plate I-1). Upon germination, a septum appeared in the center of the conidia, and germ tubes grew out from one or both cells of the conidium. The appressoria tended to develop on the sutures between epidermal cells (Plate I-1, 2).

Infection pegs emerging from the appressoria were observed 3~4 days after inoculation. The infection peg was thin, and hyphae developed gradually to their normal size within the host cells (Plate I-3). Penetration through stomatal openings was rare.

The fungus spread both inter- and intracellularly through the seed cap (Plate I-4, 5, 9). Infected cells eventually collapsed, and lesions became visible about 5 days after inoculation (Plate I-4, 6). Five to seven days after inoculation, sporulation was observed on the seed caps, anthers and perianthes.

In cross sections of the flowers, 11~14 days after inoculation, seed caps including apical pore were filled with hyphae (Plate I-5, II-3), which reached the fruit cavity and covered all over the inner surface of the pericarp. The hyphae also invaded the developing true seed causing tissue collapse. True seeds died before maturation consequently (Plate I-7, 8, II-1).

Stage 2 (Infection at the late stage of flowering)

Whereas seed balls have developed almost completely at this stage, true seeds often remained immature leaving cavity within seed balls.

Mycelia were frequently observed on the surface of the seed balls (Plate II-4) and less frequently within seeds, although these seeds could mature. A few mycelia existed in the periphery of the apical pore (Plate II-5, 6) and under the seed coat (Plate II-7, 8). The invasion of the pathogen from the basal pore or peripheral zone was not observed.

DISCUSSION

Sugar beet flowers and seed balls were susceptible to infection by *C. dematium* f. *spinaciae* at all the developmental stages. However, there was a close relationship between disease severity

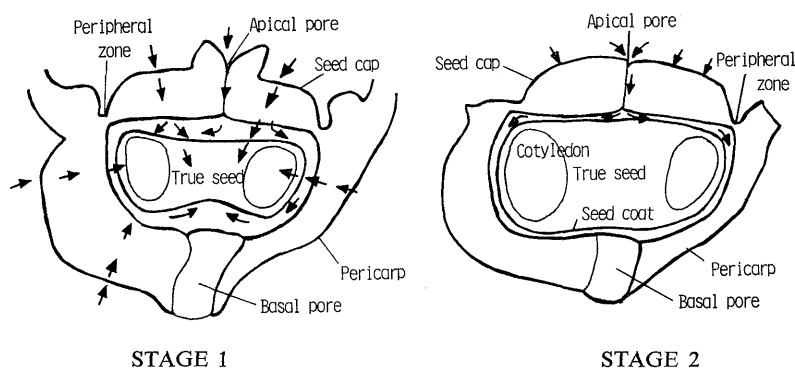


Fig. 1. Diagram of a transverse section of sugar beet seed ball. Arrows indicate the possible routes of invasion of *Colletotrichum dematium* f. *spinaciae*.

and infection stage. The pathogen easily penetrated seed caps through cuticle at the early stage of flower development (7 days after pollination, stage 1), resulting in either plant death or seed contamination. This mode of infection was in accordance with other anthracnose^{11,13}. Invasion through stomata does not seem to be common. At the late stage of flower development (21 days after pollination, stage 2), however, the pathogen could hardly penetrate the tissues and remained only on the surface. Present observations were summarized in Fig. 1. These findings together with previous one³) indicate that the pathogen was seed-borne.

The tissues surrounding the true seed become impervious with time⁸) and could form a barrier to fungal invasion. Openings which allow the air and water to enter on germination can be entrances for the pathogens, e.g. basal pore in the pericarp¹²), peripheral zone forming the slit between the seed cap and pericarp⁶) and apical pore in the seed cap⁷). El-Nashaar and Bugbee⁷) actually confirmed that *Phoma betae* invades seeds via these three openings. In the case of anthracnose, however, profuse hyphae covering the apical pore inside the seed cap at both stages (Plate II-3, 5 and 6), together with observations that symptoms first develop on the seed cap, indicate that the apical pore serves as the entrance for the pathogen.

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和 文 摘 要

築尾嘉章・杉本利哉：テンサイ花器（球果）に対する炭そ病菌の感染過程

テンサイ花器に対する炭そ病菌の感染過程を光学および走査型電子顕微鏡で観察した。宿主への侵入はクチクラ貫入により接種後 3~4 日目に起こった。菌糸は細胞間および細胞内を伸長し、接種後 5 日目には病斑が形成された。開花初期に本菌に感染すると菌糸は未熟な蒴蓋などから容易に侵入でき、細胞内に充満した。激発すると菌糸はさらに生育を続け蒴蓋と果皮にとり囲まれた真正種子の内部にまで到達し、結実を妨げた。開花後期に感染した場合は、菌糸は球果表面に多くみられたが、内部ではほとんど観察されず球果は結実した。しかし蒴蓋自然開口部周辺や真正種子の種皮下に少数の菌糸が観察され、本菌は感染時期をとわず球果内部に侵入していることが確認された。以上の結果から、テンサイ炭そ病菌は種子伝染性であると判定された。

Explanation of plates

Plate I, 1~8 and Plate II, 1~3: Scanning electron micrograph and light micrograph of sugar beet flowers (seed balls) infected with *Colletotrichum dematium* f. *spinaciae* 7 days after pollination (stage 1).

Plate II, 4~8: Infected 21 days after pollination (stage 2).

Plate I.

1. Germinated conidia of *C. dematium* f. *spinaciae* having a septum in the middle of the spores and appressorium at the tip of the germ tube on the seed cap. Three days after inoculation (scale: 10 μ m).
2. Close up of an appressorium (scale: 10 μ m).
3. Appressorium on penetration hypha, 4 days after inoculation.
4. Cross section of seed cap, 5 days after inoculation.
5. Cross section of seed cap, 11 days after inoculation.
6. Leaf surface with a lesion, 5 days after inoculation. Note the penetration hypha and an appressorium.
7. Cross section of heavily infected flower with mycelium covering embryo, 14 days after inoculation (scale: 1,000 μ m).
8. Cross section of heavily infected flower having penetration hyphae within the true seed, 14 days after inoculation.
9. Pericarp tissue with intracellular hyphae. Note hyphae passing from cell to cell.

Plate II.

1. Close up of immature seed ball. Acervuli were formed inside the seed coat, 14 days after inoculation (scale: 1,000 μ m).
2. Inner view of healthy seed cap with an apical pore (scale: 100 μ m).
3. Inner view of infected seed cap with hyphae covering the entire inner surface, 14 days after inoculation (scale: 100 μ m).
4. Outer view of seed cap with a stigma (star-shaped). Note the hyphae creeping on the stigma (scale: 1,000 μ m).
5. Cross section of the seed ball, true seed developing within the fruit cavity between the pericarp and seed cap. Note the apical pore (arrow) of the seed cap (scale: 1,000 μ m).
6. Close up of the apical pore of Plate II-5. Note hyphae (scale: 10 μ m).
7. Cross section of the seed ball. Note seed organs completely differentiated (scale: 1,000 μ m).
8. Close up of Plate II-7. Penetration hyphae (arrow) can be seen beneath the seed coat (scale: 100 μ m).

Abbreviations used in plates: A: appressorium, C: conidium, CO: cotyledon, H: hyphae, SC: seed cap, TS: true seed, P: pericarp.

Plate I

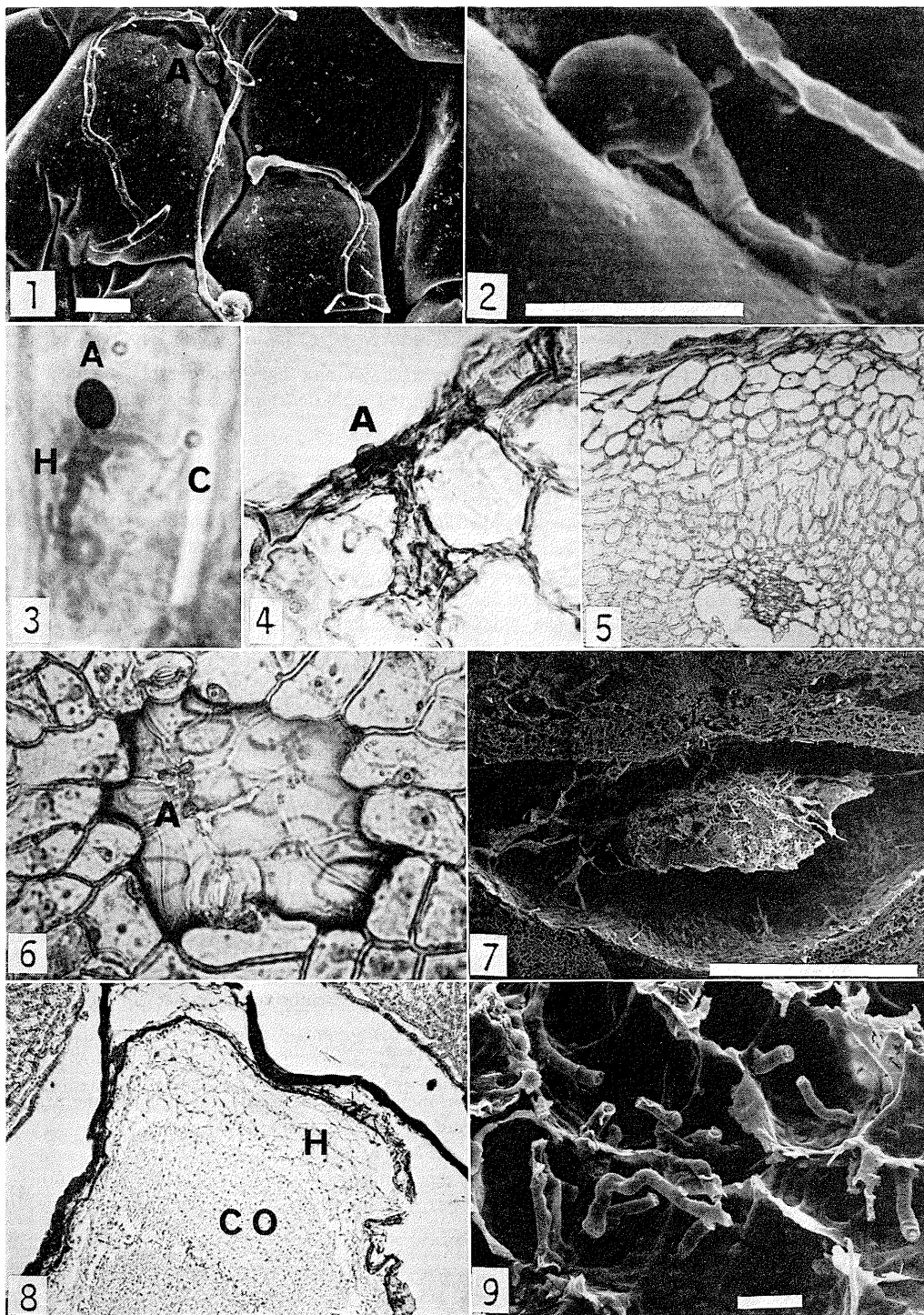


Plate II

